

**Citation:**

Motard-Belanger A, Charest A, Grenier G, et al. Study of the effect of trans fatty acids from ruminants on blood lipids and other risk factors for cardiovascular disease. *Am J Clin Nutr.* Mar 2008; 87 (3): 593-599.

**PubMed ID:** [18326596](#)

**Study Design:**

Double-blind, randomized, crossover controlled trial

**Class:**

A - [Click here](#) for explanation of classification scheme.

**Research Design and Implementation Rating:**

POSITIVE: See Research Design and Implementation Criteria Checklist below.

**Research Purpose:**

To compare the effects of ruminant derived trans fatty acids and industry derived trans fatty acids on plasma LDL concentrations and other cardiovascular disease risk factors in healthy subjects.

**Inclusion Criteria:**

Subjects were:

- Men
- Non-smokers
- 18-65 years old
- Body mass index (BMI; in kg/m<sup>2</sup>) between 18 and 30.

**Exclusion Criteria:**

Subjects were excluded if:

- Presence of a monogenic dyslipoproteinemia or any diagnosed endocrine disorder
- The use of any medication including those known to affect lipid metabolism
- The presence of a chronic, metabolic or acute disease
- Significant weight change within the six months before the experiment
- Men with food allergies, with an aversion to foods included in the experimental diets
- Alcohol consumption of more than two drinks per day.

**Description of Study Protocol:****Recruitment**

48 healthy men were recruited in the Québec City area to participate in the study.

## **Design**

This was a double-blind, randomized, crossover controlled study according to a Latin square design, in which

## **Dietary Intake/Dietary Assessment Methodology**

- Specific vegetable and animal oils and fat were incorporated in each diet to minimize differences in the amounts of saturated fatty acids (SFA) and unsaturated fatty acids between treatments. As a result, the four experimental fats used to formulate the diets contained relatively comparable amounts of every major type of SFA, monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA).
- All diets were identical in terms of menus, calories and macronutrient composition, with the exception of TFA sources and concentrations
- On average, the four experimental diets were formulated to provide 50% of daily calories from carbohydrate, 14% from protein, and 37% from fat
- The ruminant trans fatty acids and industrial trans fatty acids provided 3.6% of daily energy intake in the high TFA diets, and the rTFA provided 1.5% of daily energy intake in the moderate rTFA diet. Finally, the control diet provided 0.8% of daily energy intake from rTFA and 0% from iTFA. The intake of SFA was similar between diets, but minor differences were observed in the intakes of MUFA and PUFA.
- Experimental diets were formulated by using NUTRITION DATA.
- A food-frequency questionnaire was used to estimate the energy intake required to keep the body weight constant. This was used to minimize body weight fluctuation during the study.
- Body weight was recorded on all weekdays just before lunch
- All meals were provided to participants. On weekdays, subjects consumed their lunch under the supervision of more than one member of the research team. Weekend meals were prepared, packaged and provided at the research place the Friday lunchtime visits. All take-home meals were provided in containers that could be heated in a microwave when necessary.
- Breakfast meal represented 20% of the daily energy intake; the lunch and dinner meals each provided 40% of the daily energy intake
- Subjects were instructed to consume their entire meals
- Subjects had free access to water and to caffeine-free diet beverages. Consumption of tea and coffee was allowed with a
- limit of two cups per d (500mL per day)
- Supplementation with vitamins and natural health products and alcohol consumption were strictly forbidden during all the trial
- Throughout the study, participants were asked to maintain their usual level of physical activity, which was evaluated by a weekly questionnaire.

## **Blinding used**

This was a double-blind study.

## **Intervention**

The four experimental diets used in the present study were:

- High in ruminant trans fatty acids (rTFA) at 10.2g per 2,500kcal

- Moderate in ruminant trans fatty acids (rTFA) at 4.2g per 2,500kcal
- High in industry trans fatty acids (iTFA) at 10.2g per 2,500kcal
- Low in industry trans fatty acids (iTFA) 2.2g per 2,500 kcal or control diet.

## Statistical Analysis

- The primary analysis compared the values of each outcome measured at the end of the four experimental diets according to a Latin square study design
- The structure of the covariance matrix for each variable (intrasubject autocorrelation across repeated measures) was taken into account in all analyses to ensure the most adequate statistical fit and power
- The Tukey adjustment was used to account for the multiple comparisons of the four diets
- Comparisons of post-diet values for each outcome are presented with and without adjustment for baseline values measured at the beginning of each dietary phase
- Carryover effects were tested by introducing terms reflecting the interaction between the sequence of treatments and the treatments per se.
- Group averages are reported as means  $\pm$  SDs unless stated otherwise
- C-reactive protein (CRP) and triacylglycerol concentrations and the ratios of total to HDL cholesterol (total: HDL cholesterol) and of apolipoprotein (apo)B to apoA1 (apoB: apoA1) were logarithmically transformed before statistical analysis
- Four CRP concentrations values  $>10\text{mg/L}$  at different time-points in different participants were excluded from analysis because they suggested the presence of bacterial infection or inflammation
- Differences were considered significant at  $P \leq 0.05$
- Data were analyzed by using the PROC MIXED procedure for repeated measures in SAS software (version 8.02; SAS, Inc, Cary, NC).

## Data Collection Summary:

### Timing of Measurements

- There were four experimental isoenergetic dietary treatments each lasting four weeks
- Plasma lipid concentrations were analyzed from fasting blood samples (after 12-hour fast), which were collected from an antecubital vein at the beginning (day one) and the end (day 26) of each experimental period
- Anthropometry and blood pressure was conducted at the beginning and at the end of each experimental diet
- A validated food-frequency questionnaire was administered to the participants at the beginning and at the middle of the study to estimate the energy intake required to keep the body weight constant.

### Dependent Variables

Assessments of the basic lipid profile and of lipoprotein-lipid concentrations by ultracentrifugation were performed according to previously described methods. Among the variables:

- Cholesterol (mmol/L)
- VLDL-C (mmol/L)
- LDL-C (mmol/L)
- HDL-C (mmol/L)
- HDL2-C (mmol/L)

- HDL3-C (mmol/L)
- TG (mmol/L)
- ApoB (g/L)
- ApoA1 (g/L)
- Total/HDL-C
- LDL-C/HDL-C
- ApoB/apoA1
- CRP (mg/L).

### Independent Variables

Dietary treatments were:

- High in ruminant TFA (10.2g per 2,500kcal)
- Moderate in ruminant TFA (4.2g per 2,500kcal)
- High in industrial TFA (10.2g per 2,500kcal)
- Low in TFA from any source (2.2g per 2,500kcal) (control diet).

### Control Variables

From TABLE 5: Physical characteristics and plasma lipid profile of the 38 subjects at baseline<sup>1</sup>

- Age (years) 32.8±15.0
- Weight (kg) 73.8±9.8
- BMI (kg/m<sup>2</sup>) 23.6±3.3
- Waist girth (cm) 81.5±9.9
- Systolic BP (mm Hg) 114.1±11.8
- Diastolic BP (mm Hg) 72.5±8.1
- Fasting glucose (mmol/L) 5.08±0.44
- Cholesterol (mmol/L) 4.32±1.03
- LDL-C (mmol/L) 2.56±0.86
- HDL-C (mmol/L) 1.25±0.23
- Triacylglycerol (mmol/L) 1.14±0.81
- <sup>1</sup>All values are mean ± SD. BP, blood pressure; C, cholesterol.

### Description of Actual Data Sample:

- **Initial N:** Recruitment: 48 men
- **Attrition (final N):** 38 men
- **Age:** 18 to 65 years
- **Ethnicity:** None specified
- **Other relevant demographics:**
  - Race, (36 white, two black)
- **Anthropometrics:**
  - Weight (kg) 73.8±9.8
  - BMI (kg/m<sup>2</sup>) 23.6±3.3
  - Waist girth (cm) 81.5±9.9
- **Location:** Quebec, Canada.

### Summary of Results:

## Key Findings

- Plasma LDL-cholesterol concentrations were significantly higher after the high- rTFA diet than after the control (P<0.05) or the moderate- rTFA (P<0.05) diet.
- Plasma LDL-cholesterol concentrations were also significantly (P<0.02) higher after the iTFA diet than after the moderate-rTFA diet, but similar to those of the control diet.
- Plasma HDL-cholesterol concentrations were significantly (P<0.02) lower after the high rTFA diet. None of the other intervention diets affected HDL-cholesterol. This reduction was attributed to the HDL2-C subfraction more than to the HDL3-C subfraction.
- All risk factors were comparable between the control and the moderate-rTFA diets. In summary, the study showed that a high intake of rTFA may lead to deleterious changes in lipid CVD risk factors, similar to those that have been attributed to TFA from industrial sources.

Modified Table: Plasma cholesterol concentrations at the end of each dietary intervention in the 38 subjects<sup>1</sup>

Variable	Dietary				Pooled SD <sup>2</sup>	P	
	Control	rTFA		iTFA		Unadjusted	Adjusted <sup>3</sup>
		Moderate	High				
Cholesterol (mmol/L)	4.77 ± 0.93	4.72 ± 0.88	4.92 ± 0.98 <sup>6</sup>	4.88 ± 0.95 <sup>6</sup>	0.45	0.009	0.004
LDL-C (mmol/L)	3.27 ± 0.80	3.22 ± 0.83	3.47 ± 0.90 <sup>5,6</sup>	3.42 ± 0.89 <sup>6</sup>	0.39	0.002	0.001
HDL-C (mmol/L)	1.25 ± 0.24	1.28 ± 0.28	1.22 ± 0.26 <sup>6</sup>	1.23 ± 0.24	0.16	0.066	0.046
HDL2-C (mmol/L)	0.59 ± 0.21	0.59 ± 0.21	0.54 ± 0.19 <sup>5</sup>	0.56 ± 0.18	0.12	0.08	0.04
HDL3-C (mmol/L)	0.66 ± 0.14	0.69 ± 0.13	0.68 ± 0.11	0.67 ± 0.13	0.16	0.34	0.32
TG (mmol/L) <sup>7</sup>	0.98 ± 0.45	0.95 ± 0.41	0.99 ± 0.43	0.97 ± 0.54	0.35	0.84	0.87
ApoB (g/L)	0.94 ± 0.23	0.91 ± 0.21	0.96 ± 0.23 <sup>6</sup>	0.94 ± 0.22 <sup>6</sup>	0.12	0.03	0.009
ApoA1 (g/L)	1.51 ± 0.19	1.53 ± 0.18	1.49 ± 0.18 <sup>6</sup>	1.52 ± 0.18	0.13	0.08	0.049
Total/HDL-C <sup>7</sup>	3.97 ± 1.16	3.86 ± 1.16	4.23 ± 1.32 <sup>6</sup>	4.16 ± 1.39 <sup>6</sup>	0.14	0.002	0.003
LDL-C/HDL-C	2.75 ± 1.00	2.67 ± 1.01	3.02 ± 1.15 <sup>5,6</sup>	2.94 ± 1.17	0.51	0.003	0.002
ApoB/apoA1 <sup>7</sup>	0.63 ± 0.21	0.60 ± 0.17	0.65 ± 0.19	0.63 ± 0.17	0.16	0.009	0.04

Author

<sup>1</sup> rTFA, trans fatty acids from ruminants; iTFA, TFA from industrial sources; C, cholesterol; TG, triacylglycerol; apo, apolipoprotein; CRP, C-reactive protein.

<sup>2</sup> Pooled SD represents the SD of the change in each variable when subjects switched from the control diet to any of the other 3 experimental diets. A greater value generally reflects a greater intra-individual variability in the response to the moderate-rTFA, high-rTFA, and iTFA diets.

<sup>3</sup> Adjusted for diet-specific baseline values.

<sup>4</sup> Mean ± SD (all such values).

<sup>5</sup> Significantly different from the control diet, P < 0.05.

<sup>6</sup> Significantly different from the moderate-rTFA diet, P < 0.05.

<sup>7</sup> These analysis were performed on log-transformed values. The apoB/apoA1 ratio was a significantly different only between diets.

## Conclusion:

The results suggest that although a high dietary intake of trans fatty acids from ruminants may adversely affect cholesterol homeostasis, moderate intakes of ruminant trans fatty acids that are well above the upper limit of current human consumption have neutral effects on plasma lipids and other cardiovascular disease risk factors.

## Reviewer Comments:

*This was a very well designed trial.*

*Main limitations were:*

- Study involved only males;
- Small sample size

## Research Design and Implementation Criteria Checklist: Primary Research

## Relevance Questions

1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	No
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	No

## Validity Questions

1.	<b>Was the research question clearly stated?</b>	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	N/A
2.	<b>Was the selection of study subjects/patients free from bias?</b>	Yes
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	Yes
3.	<b>Were study groups comparable?</b>	N/A
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	Yes
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes

3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
<b>4.</b>	<b>Was method of handling withdrawals described?</b>	<b>Yes</b>
4.1.	Were follow-up methods described and the same for all groups?	Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	N/A
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	Yes
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
<b>5.</b>	<b>Was blinding used to prevent introduction of bias?</b>	<b>Yes</b>
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	Yes
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
<b>6.</b>	<b>Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?</b>	<b>Yes</b>
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A

6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
<b>7.</b>	<b>Were outcomes clearly defined and the measurements valid and reliable?</b>	<b>Yes</b>
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	Yes
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes
7.7.	Were the measurements conducted consistently across groups?	Yes
<b>8.</b>	<b>Was the statistical analysis appropriate for the study design and type of outcome indicators?</b>	<b>Yes</b>
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	Yes
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes
8.6.	Was clinical significance as well as statistical significance reported?	Yes

8.7.	If negative findings, was a power calculation reported to address type 2 error?	N/A
<b>9.</b>	<b>Are conclusions supported by results with biases and limitations taken into consideration?</b>	Yes
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	Yes
<b>10.</b>	<b>Is bias due to study's funding or sponsorship unlikely?</b>	Yes
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	Yes